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MICROSCOPY.

Methods for the Preservation of Marine Organisms Employed at the Naples Zoological Station.—Unfortunately for our students, especially those living inland and depending largely for their knowledge of marine forms upon dried or preserved specimens in museums, the old-fashioned methods of throwing any material which the collector may find into a jar of alcohol without further attention, or else drying it in the sun, are still almost the only ones made use of for the preservation of museum specimens. The result is that the majority of forms which the student has for study are either dried skeletons, or shrivelled up monstrosities giving no idea whatever of the actual appearance of the creatures supposed to be represented by them. How many college museums possess a specimen of coral showing in any recognizable form the polyps by which the skeleton coral was formed? Or how many have even a satisfactorily prepared Lamellibranch?

There are, however, in this country, a few collections which show a marvellous improvement in their manner of preparation, and which have been purchased for the Naples Zoological Station, whose conservator, Salvatore Lo Bianco, has, for several years, been devoting himself to the discovery of the best methods for the preservation of the form and color of the marine animals occurring in the Mediterranean. Until the present, however, his discoveries have not been made common property, except in the few cases where the most successful methods of preserving certain forms have been published in connection with accounts of their structure. The last number of the *Naples Mittheilungen*,[1] however, contains a full description, by Lo Bianco, of the methods found most successful for the preservation of the various forms which occur at Naples, and which are undoubtedly applicable to the similar forms found upon our own coast. An abstract of these methods is given in the following pages, in the hope that they may be found useful by the museum curators of this country, and that their application may result in the much-needed improvement of the appearance of the specimens found in the majority of our college museums.

It must be fully understood, however, that much depends upon the skill of the preparator, and that want of care and patience will frequently counteract all the advantages to be derived from a good

[1] Mitth. a. d. zool. Station zu Neapel, Bd. IX., Heft III.

method. All who have had the opportunity of examining specimens prepared by Lo Bianco can appreciate readily the great advantages which may result from the careful application of his methods, and can perceive how greatly we are indebted to him and to Professor Dohrn for their publication.

Alcohol is, of course, indispensable as preservative fluid, but certain precautions are necessary in its use. Except in a very few cases it is unnecessary to use it in its full strength, 70% being quite sufficient for preservation, and producing much less contraction and fragility in delicate organisms. Strong alcohol should be reduced with distilled water to the desired strength, ordinary spring water frequently containing a sufficient amount of carbonate of lime and other substances in solution to give a cloudy precipitate, after a time, which may effectually destroy the appearance of a specimen. Furthermore, delicate organisms should first be placed in weak alcohol (35 to 50%) for from 2 to 6 hours, the changing of the fluids being effected by a siphon, a small quantity of the weak alcohol being withdrawn and stronger added, until finally the desired strength is obtained. *With delicate and gelatinous structures the increase in the strength of the alcohol should be as gradual as possible.*

In many cases it is necessary to use a hardening or fixing reagent before the final consignment to alcohol, which is principally useful as a preservative. The most useful fixing reagents, according to Lo Bianco, are the following:

Chromic Acid.—1 per cent. in fresh water. Objects should not remain in this fluid longer than is necessary to fix them, as they are apt to become brittle. Subsequently they should be well washed with distilled water to prevent the formation of a precipitate when placed in alcohol, and also to prevent their taking on too green a tinge from the reduction of the acid.

Acetic Acid, concentrated, kills rapidly contractile animals, but must be used with caution, as it produces a softening of the tissues if they are subjected for too long a time to its action.

Osmic Acid.—1 per cent. solution, hardens gelatinous forms well, and preserves their transparency, but its prolonged action renders the object brittle and gives it a dark brown tint. Objects hardened in it should be well washed in distilled water before being placed in alcohol.

Lactic Acid.—1 part to 1000 parts sea-water fixes larvæ and gelatinous forms well.

Corrosive Sublimate.—Saturated solution in fresh or sea-water; may be used either hot or cold. It acts quickly, and preserves admirably for histological purposes. It is especially good combined with copper sulphate, acetic acid, or chromic acid. Objects hardened in it should be subsequently well washed in distilled water and in iodized alcohol (the recipe for which is given below), to remove all traces of the sublimate, which in alcohol crystallizes out in the tissues of the organisms and so injures the preparation.

Bichromate of Potassium.—5 per cent. solution in distilled water hardens gelatinous organisms slowly, without rendering them fragile. It gives, however, a precipitate in alcohol, and discolors the specimen. The discoloration, however, may be removed by adding to the alcohol a few drops of concentrated sulphuric acid.

Copper Sulphate.—5 per cent. or 10 per cent. solution in distilled water, used either alone or in combination with corrosive sublimate, kills larvæ and delicate animals without distortion. The objects should be subsequently repeatedly washed with water to remove all traces of of the salt, otherwise crystals will form when the object is placed in alcohol.

Various combinations of these reagents are especially useful, and some of those most serviceable are given here:

Alcohol and chromic acid.

70 per cent. alcohol	equal parts.
1 per cent. chromic acid	

Alcohol and hydrochloric acid.

50 per cent. alcohol	100 c.c.
Hydrochloric acid, concentrated	5 c.c.

Iodized alcohol.

35 per cent. or 70 per cent. alcohol	100 c.c.
Tincture of iodine	2.5 c.c.

Chrom-acetic acid, No. 1.

1 per cent. chromic acid	100 c.c.
Concentrated acetic acid	5 c.c.

Chrom-acetic acid, No 2.

Concentrated acetic acid	100 c.c.
1 per cent. chromic acid	10 c.c.

Chrom-osmic acid.

1 per cent. chromic acid	100 c.c.
1 per cent. osmic acid	2 c.c.

Chrom-picric acid.

1 per cent. chromic acid	equal parts.
Kleinenberg's picrosulphuric acid	

Copper sulphate and corrosive sublimate.

10 per cent. solution of copper sulphate	100 c.c.
Saturated solution of corrosive sublimate	10 c.c.

Potassium bichromate and osmic acid.

5 per cent. solution of potassium bichromate	100 c.c.
1 per cent. osmic acid	2 c.c.

Corrosive sublimate and acetic acid.

Saturated solution of corrosive sublimate	100 c.c.
Concentrated acetic acid	50 c.c.

Corrosive sublimate and chromic acid.

Saturated solution of chromic sublimate	100 c.c.
1 per cent. chromic acid	50 c.c.

Frequently great difficulty is experienced in killing an animal without producing a considerable amount of contraction, and in the case of elongated forms, such as Nemertean and other worms, without causing them to coil up or become twisted. To avoid this, it is expedient to narcotize the animals before killing them, and for this purpose Lo Bianco recommends immersion in weak alcohol. He uses generally a mixture of sea-water 100 c.c. and absolute alcohol 5 c.c. In other cases 70 per cent. alcohol may be carefully poured upon the water in which the specimen lies, so that it forms a layer at the surface. It will gradually mix with the subjacent water, and in the course of a few hours will narcotize the animal, so that it may be treated with fixing reagents without fear of contraction.

Chloral hydrate, 1 to 2 parts to 1000 parts sea-water, is also efficient as a narcotizing agent, and has the advantage of allowing a recovery of the animal, if there should be necessity for it, by placing it in fresh sea-water.

For some sea-anemones tobacco smoke is useful, the smoke being conducted by a V-shaped tube into a bell-jar covering the vessel of sea-water in which is the anemone.

Certain of these reagents will prove most satisfactory with some animals, others with others. Lo Bianco details the best method for treating the various forms in a second portion of his paper, and an account of some of his methods of procedure, so far as they concern forms which resemble those found upon our coast, may now be presented.

Sponges.—Direct immersion in 70 per cent. alcohol, with subsequent renewal of the fluid, is recommended for the majority of forms. To avoid contraction in the case of the Halisarcidæ they should be left for half an hour in 1 per cent. chromic acid, or in concentrated solution of corrosive sublimate for fifteen minutes. To prepare dried specimens the sponges should be washed in fresh water for a few hours, and then allowed to remain in ordinary alcohol for a day, after which they may be dried in the sun.

Anthozoa.—The first care must be to place the forms belonging to this group in fresh salt-water, to allow them to expand, a result which may not be obtained until the following day in some cases. Alcyonarians should be killed with chrom-acetic solution No. 2, withdrawing the water in which they lie until there is left just enough to cover them, and then adding a volume of the chrom-acetic solution double that of the sea-water. The animals should be removed from this mixture the moment they are killed, since the acid will quickly attack the calcareous spicules, which are important for the identification of the Alcyonaria, and placed in 35 per cent. or 50 per cent. alcohol, it being well to inject the alcohol into the mouths of the polyps to keep them freely expanded. The preparation should finally be preserved in 70 per cent. alcohol.

Regarding the Actinians no definite rule for preservation can be given. Much of the success of the preparation depends on the form employed, some species contracting much less readily and less perfectly than others. Some may be killed in a fair condition by pouring over them when expanded boiling corrosive sublimate, and then, before consigning them to alcohol, treating for a few minutes with one-half per cent. chromic acid. This method may be employed with small forms such as *Aiptasia*. Narcotization may be tried with others. For this purpose remove from the vessel in which the animals are contained two-thirds of the sea-water, and replace it with a 2 per cent. solution of choral hydrate. After a few minutes the fluid is again removed, and cold concentrated sublimate solution is poured in. Tobacco smoke, in some cases, as with *Adamsia*, will act satisfactorily, if followed with vapor of chloroform for two to three hours, after which the animals may be killed in chrom-acetic, solution No. 2, and hardened in one-half per cent. chromic acid.

Edwardsia may be narcotized by gradually adding 70 per cent. alcohol to the sea-water in which they are, and subsequently may be killed with hot corrosive sublimate.

Cerianthus should be killed with concentrated acetic acid, placing it as soon as possible in weak alcohol, in which it should be suspended so that the tentacles may float freely, if necessary disentangling them.

Corals should be allowed to expand fully, and should then be killed with boiling solution of corrosive sublimate and acetic acid used in volume equal to that of the sea-water containing the coral. The colony should then be transferred to 35 per cent. alcohol, some of this fluid being injected into the mouth of each polyp. The injection should be repeated at every change of the alcohol, and the specimens should be preserved in 70 per cent. alcohol, after washing them well in iodized alcohol.

Hydromedusæ.—For the hydroid colonies the best fixing reagent is hot corrosive sublimate. The smaller Tubularian medusæ should be killed either in the mixture of corrosive sublimate and acetic acid, or in Kleinenberg's picrosulphuric acid. Larger forms may be fixed with concentrated acetic acid, and then allowed to fall into a tube containing the alcohol and chromic acid mixture, in which they are gently agitated and allowed to remain for fifteen minutes, after which they should be transferred to 35 per cent. alcohol and gradually carried to 70 per cent.

Small Campanularian medusæ, *e.g.*, *Eucope* and *Obelia*, may be killed in the mixture of copper sulphate and corrosive sublimate. *Æquorea* should be killed with concentrated acetic acid, and immediately transferred to chrom-osmic mixture for fifteen to thirty minutes. The same method answers for *Cunina*, while *Liriope* should be treated at once with chrom-osmic for five to twenty minutes.

Scyphomedusæ are best fixed with 1 per cent. osmic acid, to the action of which they are subjected until they assume a pale brown tint. They should then be thoroughly washed with fresh water before being placed in 35 per cent. alcohol, and should be finally preserved in 70 per cent.

Siphonophores.—The forms of this group should be preserved soon after capture, and specimens in good condition should be selected. *Agalma* and similar forms should be killed in the mixture of copper sulphate and sublimate, which should be used in volume equal to or double that of the sea-water in which the animal floats. The mixture should be poured in rapidly, and *not* over the animal. When killed, the specimen should be carefully lifted upon a large horn spatula, and transferred to 35 per cent. alcohol for a few hours, and then placed in 70 per cent. It is recommended to preserve the animals in tubes just large enough to contain the specimens and placed within a second larger tube. In this way evaporation of the alcohol is prevented, and also injury of the specimen from movements of the liquid is avoided.

Physalia should be placed in a cylinder filled with sea-water, the animal being lifted by the pneumatophore. When well expanded it is killed by pouring over it the sublimate and acetic acid mixture (one-quarter the volume of the sea-water), and when dead is transferred to a cylinder containing one-half per cent. chromic acid, and then after twenty minutes to 50 per cent. alcohol, and finally to 70 per cent.

Velella may be killed with chrom-picric or sublimate and chromic acid mixture, and after a few minutes should be transferred to weak alcohol. *Porpita* may be fixed by dropping Klienenberg's picro-sulphuric acid into the vessel in which it is contained, and when the blue color commences to change to red it should be transferred to Kleinenberg's fluid, and after fifteen minutes to weak alcohol.

Diphyes may be killed expanded by hot corrosive sublimate.

Ctenophora may be killed by throwing them into the chrom-osmic mixture, where they should remain for fifteen to sixteen minutes, according to the size, and then gradually passing them through alcohol to 70 per cent. A mixture composed of

Pyroligneous acid, concentrated, ...	1 vol.
Corrosive sublimate solution, ...	2 vols.
One-half per cent. chromic acid, ...	1 vol.

is also recommended as a fixative.

Echinodermata.—Starfish may be prepared with the ambulacral feet in full distension by allowing them to die in 20 to 30 per cent. alcohol.

Echinoids should be placed in a small quantity of water, and killed with chrom-acetic mixture No. 2, being removed from it as quickly as possible, as the acid corrodes the test. To preserve the internal parts it is necessary to make two opposite openings in the test, so that the alcohol may penetrate the interior readily.

Holothurians, such as *Thyone* and *Cucumaria*, after the tentacles are fully expanded, should be seized a little below the bases of the tentacles by forceps, using a slight pressure, and the anterior portion of the body should then be immersed in concentrated acetic acid. Alcohol (90 per cent.) should then be injected into the mouth, and the specimens placed in 70 per cent. alcohol. The injection should be repeated each time the alcohol is changed.

Synapta should be fixed by immersion in a tube containing a mixture of equal parts of sea-water and ether (or chloroform), where they remain completely expanded. They should then be washed for a short time in fresh water, and passed into alcohol, taking care to increase the strength of this very gradually.

Vermes.—Cestodes, Trematodes, Turbellaria, as well as Nemathelminthes, are most satisfactorily killed with corrosive sublimate, either cold or hot. *Sagitta,* however, succeeds best in copper sulphate and sublimate or chrom-osmic mixture.

Nemerteans should be narcotized in a solution of chloral hydrate in sea-water 1 per cent., where they should remain for 6 to 12 hours. They are then to be hardened in alcohol.

Gephyreans may be narcotized with 1 per cent. solution of chloral hydrate in sea-water, or in alcoholized sea-water, 3 to 6 hours; or else may be killed at once in one-half per cent. chromic acid, which last method may also be applied to *Hirudinei.*

Chætopods are best narcotized in sea-water containing 5 per cent. of absolute alcohol, or by adding gradually to the surface of the sea-water in which they are contained a mixture of glycerine 1 part, 70 per cent. alcohol 2 parts, and sea-water 2 parts, hardening them subsequently in alcohol. *Chætopterus* is best killed with 1 per cent. chromic acid, in which they should remain for half an hour; while the Hermellidæ, Aphroditidæ, and the Eunicinæ may be killed in cold corrosive sublimate. Some of these, such as *Diopatra,* may, however, be narcotized in alcoholized sea-water. Serpulidæ, before treatment with corrosive sublimate, should be narcotized in 1 per cent. chloral hydrate, which causes them to protrude wholly or partly from their tubes.

Crustacea.—Cladocera, Copepods and Schizopods may be killed in corrosive sublimate, dissolved in sea-water. Ostracodes may be thrown at once into 70 per cent. alcohol. Cirripedes die expanded in 35 per cent. alcohol, and if some specimens contract it is easy to draw out the cirrhi with forceps. Amphipods and Isopods may pass directly into 70 per cent. alcohol, except the Bopyrids and Entoniscids, which should be killed in the mixture of equal parts of 90 per cent. alcohol and sublimate solution.

To avoid the casting off of the appendages of the Decapods they should be allowed to die in fresh water, care being taken not to allow them to remain in it longer than is necessary, as it causes a distortion of the membranous appendages.

Pycnogonids will die in one-half per cent. chromic acid with the appendages fully extended.

Mollusca.—Lamellibranchs, Prosobranchs and, Heteropods should be narcotized in alcoholized sea-water. To avoid the closure of the valves of Lamellibranchs on immersion in 70 per cent. alcohol, little plugs of wood should be placed between the margins of the valves. The same result may be effected in the case of Prosobranchs by tying the internal edge of the operculum to the shell.

Of the Opisthobranchs the æolidæ may be best preserved by pouring over them concentrated acetic acid in volumes equal to or double that of the sea-water containing them. Dorids should first be narcotized by gradually adding 70 per cent. alcohol to their sea-water, and then killed with concentrated acetic acid or boiling sublimate. The larger forms may be killed in 1 to 5 per cent. chromic acid.

Pteropods are preserved well in Pereny's Fluid for fifteen minutes, whence they are passed to 50 per cent. alcohol. Gymnosomatous forms should be first narcotized with 1 per cent. chloral hydrate, and then killed in acetic acid or sublimate.

Decapod Cephalopods may be fixed directly in 70 per cent. alcohol, making an opening on the ventral surface to allow the alcohol to reach the internal parts.

Bryozoa.—The genera *Pedicellina* and *Loxosoma* may be left for an hour in 1 per cent. chloral hydrate, and then killed with cold corrosive sublimate, washing them immediatety afterwards. Some species of *Bugula* give good results when the expanded animals are suddenly killed by pouring over them hot corrosive sublimate. With other forms it is sometimes possible to preserve them well expanded by adding 70 per cent. alcohol gradually to the surface of the water in which they are, or by narcotizing first in weak chloral hydrate or in alcoholized sea-water. The results are, however, uncertain, and depend largely on the skill of the preparator.

Brachiopoda may be treated in the same manner as Lamellibranchs.

Tunicates.—*Clavellina*, *Perophora*, and *Molgula* may be killed with the orifices expanded by immersing them in 1 per cent. chloral hydrate for 6 to 12 hours. They should then be killed in chrom-acetic mixture, No. 2, and quickly transferred to 1 per cent. chromic acid, injecting some of the fluid into the body. After half an hour they should be transferred in 35 per cent. alcohol, the injection being repeated, and finally to 70 per cent. Other simple forms may be treated in the same manner, or may require the 2 per cent. solution of chloral hydrate, or may be killed by pouring a little 1 per cent. chromic acid on the surface of the water in which they are, subsequently hardening in 1 per cent. chromic acid. The method recommended for *Perophora* may be employed for compound Ascidians, using, however, corrosive sublimate instead of the chrom-acetic mixture.

Salpæ vary considerably in consistency, according to the species, and different methods are consequently required. The denser forms, such as *S. zonaria*, should be placed in a mixture of 100 c.c. fresh water and 10 c.c. concentrated acetic acid, where they should remain for fifteen minutes. They should then be washed in fresh water for ten minutes, and pass gradually into alcohol. Less dense forms, such as *S. democratica mucronata*, may be fixed in chrom-acetic mixture, No. 1, and then passed directly into fresh alcohol; while the soft forms, such as *S. pinnata* and *maxima*, should be placed in chrom-osmic mixture for 15 to 60 minutes, then washed in fresh water, and transferred to weak alcohol.

Fishes.—*Amphioxus* will die with the buccal cirrhi distended in sea-water alcoholized to 10 per cent. They should then be transferred to 50 per cent. alcohol, and gradually to 70 per cent.

Other forms may be preserved in alcohol (70 per cent.), taking care to make a ventral incision, and also to inject the alcohol and renew it frequently at first. If it is wished to preserve some of the larger Selachians for some months in order to prepare at leisure the skeleton, the intestines should be removed, and the animals placed in a 10 per cent. solution of salt.

Elasmobranch embryos may be fixed in corrosive sublimate, leaving them in the solution for 5 to 15 minutes, afterwards washing well in iodized alcohol. Embryos of *Torpedo* with the yolk were preserved by immersing them in a mixture of equal parts of 1 per cent. chromic acid and corrosive sublimate for fifteen minutes, and then transferring to alcohol.

Transparent fish eggs may be preserved for the purpose of demonstration by subjecting them for a few minutes to the action of the alcohol and hydrochloric acid mixture, and then transferring them to pure alcohol.—PLAYFAIR M'MURRICH.